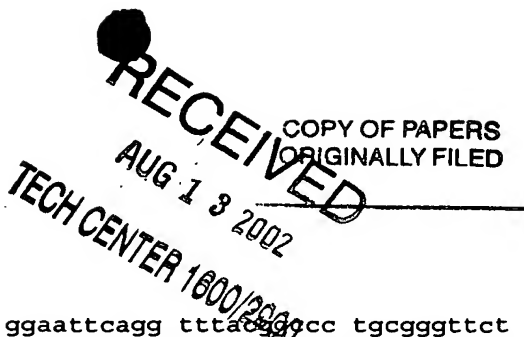




Figure 1



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301 taagacttgc ccaggaaaat gaagtggatt ttattttgtt aggtgggtgat ctttttcatg  
361 aaaataagcc ctcaaggaaa acattacata cctgcctcga gttattaaga aaatattgta  
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481 gtaagtttcc atgggtgaac tatcaagatg gcaacctcaa catttcaatt ccagtgttta  
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2401 actccataag taagaaattt ctagtccaca gacatacaat agcattgatt caccttgttt  
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Figure 2

SEQ ID NO:2

```

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601 tstrsrnskt avsasrnmsi idafkstrqq psrnvttkny sevievdesd veedifptts
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# Schematic Representation of Mre11 Activity

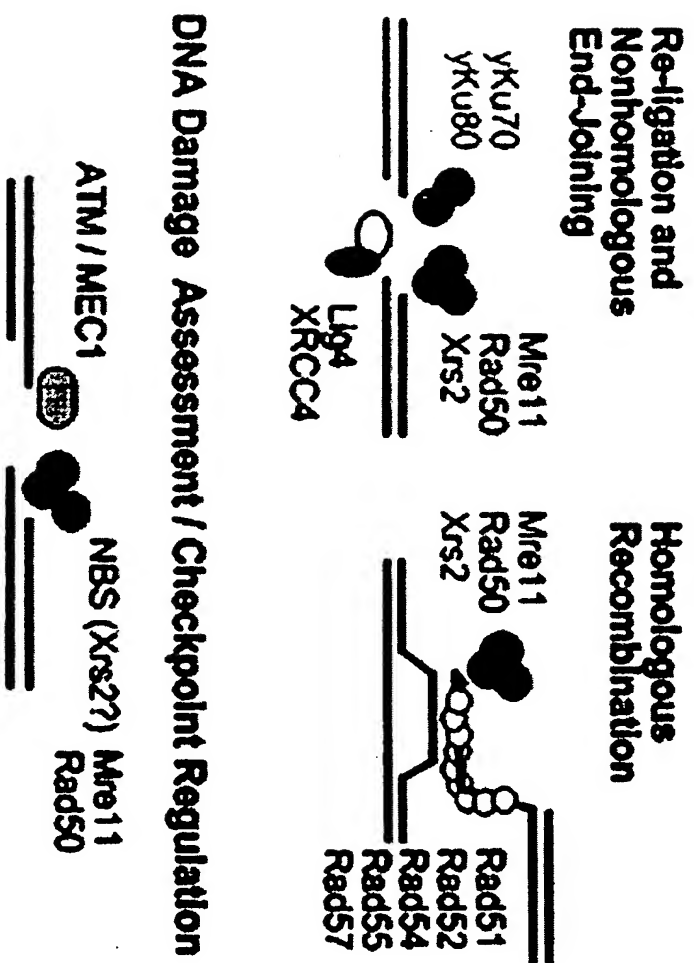


FIG. 3

# Dominant Negative Mutants Generated for Target Validation Studies

Two inactivating mutants were generated analogous to catalytically inactivating mutations in the yeast MRE11:

H217Y (MCB1998 Jan.18(1):260-68 )  
H129N (MCB1999 Jan.19(1):556-66 )

Both histidines are thought to form part of the  $Mn^{2+}$  coordination site (7 histidines coordinate 2  $Mn^{2+}$  ions) in the catalytic core of the protein. H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

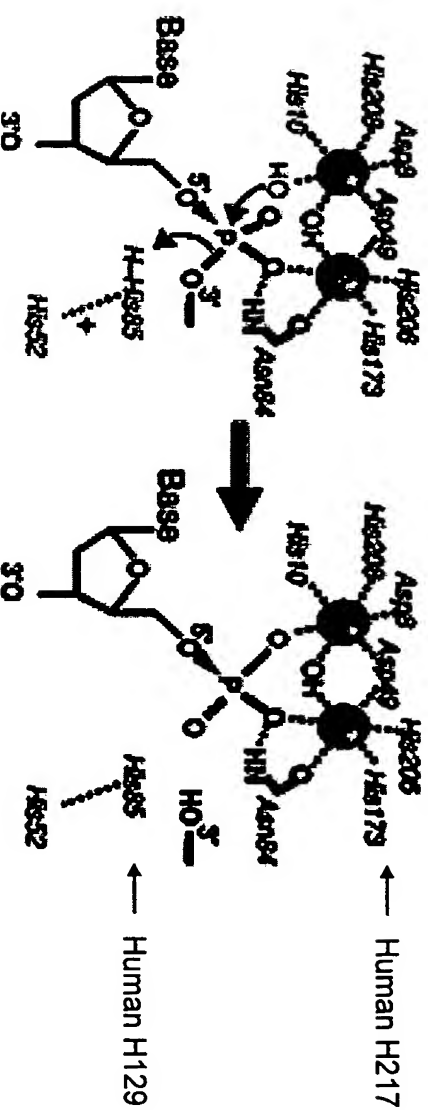
```
hMRE11      9  DENTFKILVATDHIHGFMEKDAKRGNDTFVTLDLILRLAQENEVDLILG GDLFHNKPS 68
              D +T +IL+ TD H+G+ E D  G+D++ T  E++ LA+ N VD ++  GDLFH NKPS
SCMRE11     5  DEDTIRILITTDNHVGVNENDPITGDDSWKTFHEVMMAKNNVDMVVGSDLFFHNKPS 64

              69  RKTILHTCLELRLKRYCMGDRPVQFEILSDQSVNFGSKFPPWVNYQDGNLNSIPVESIHGN 128
              +K+L+ L+ LR  CMGD+P + E+LSD S  F + +F  VNY+D N NI SIPVF I GN
              65  KKSLYQVLKTLRLCCMGDKPCELELLSDPSQVFHYDEFTNVNVEDPNENISIPVEGISGN 124
              *
              129  HDDPTGADALCALDILSCAGFVNHFGRSMVSVEKIDISPVLLQKGSTKIALYGLGSIPIER 188
              HDD +G  LC +DIL  G +NHFG+ +  +KI + P+L QKGSTK+ALYGL ++ DER
              125  HDDASGDSLLCPMDILHATGLINHFGKVIKESDKIKVVPPLLQKGSTKIALYGLAVERDER 184
              *
              189  LYRMEVNNKVTMLRPKEDENSWENLFLVI HQNRSKHGSTNFIPEQFLDDFIDLVINGHEHE 248
              L+R F + VT  P  E  WENL + HQN + H +T F+PEQFL DF+D+VINGHEHE
              185  LFRTEKDGCVTFEVPFTMEGEMENLMCV HQNHTGHTNTAFELPEQFLPDLDMVINGHEHE 244

              249  CKIAPTNEQQLFYISQPGSSVVTSLSPGEAVKKHVGLLRK -GRKMMHKIPLHTVROF 307
              C      N + F + QPGSSV TSL  EA  K+V +L IK G  M  IPL T+R F
              245  CIPNLVHNPIKNEVDLQPGSSVATSLCEAEAOQPKYVFILDIKYGEAPKMTPIPLETIRTF 304
```

FIG. 4

# Representation of Active Site of *P. furiosus* Mre11



$Mn^{2+}$  coordination site 7 histidines coordinate 2  $Mn^{2+}$  ions in the catalytic core of the protein.

H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

Mutations Generated for Dominant Negative Studies correspond to:

<i>P. furiosus</i>	Human
H173	H217
H85	H129

FIG. 5

# Summary of Target Validation Studies: MRE11

## Dominant negative studies

	Antiproliferative Activity						
	Tumor		Normal				
	A549	Hela	PC3	H1299	HMEC	HUVEC	PrEC
<b>Wt</b>							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
<b>H217Y</b>							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
<b>H129N</b>							
GFP-fusion	++	++	-/+	-/+	-	-	-
IRES GFP	+	-	nd	nd	-	-	nd

**Antisense:** **A549**  
**inconclusive**

( + indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)

**FIG. 6**

# Summary of Target Validation Studies: MRE11

Dominant negative studies			
	Chemosensitization Activity		
	Tumor A549	Hela	HMEC
Wt			
GFP-fusion	-	-	-
H217Y			
GFP-fusion	++	++	-

FIG. 7

# Overexpression of GFP-Fused MRE11 H129N is Antiproliferative in A549 and HeLa Cells

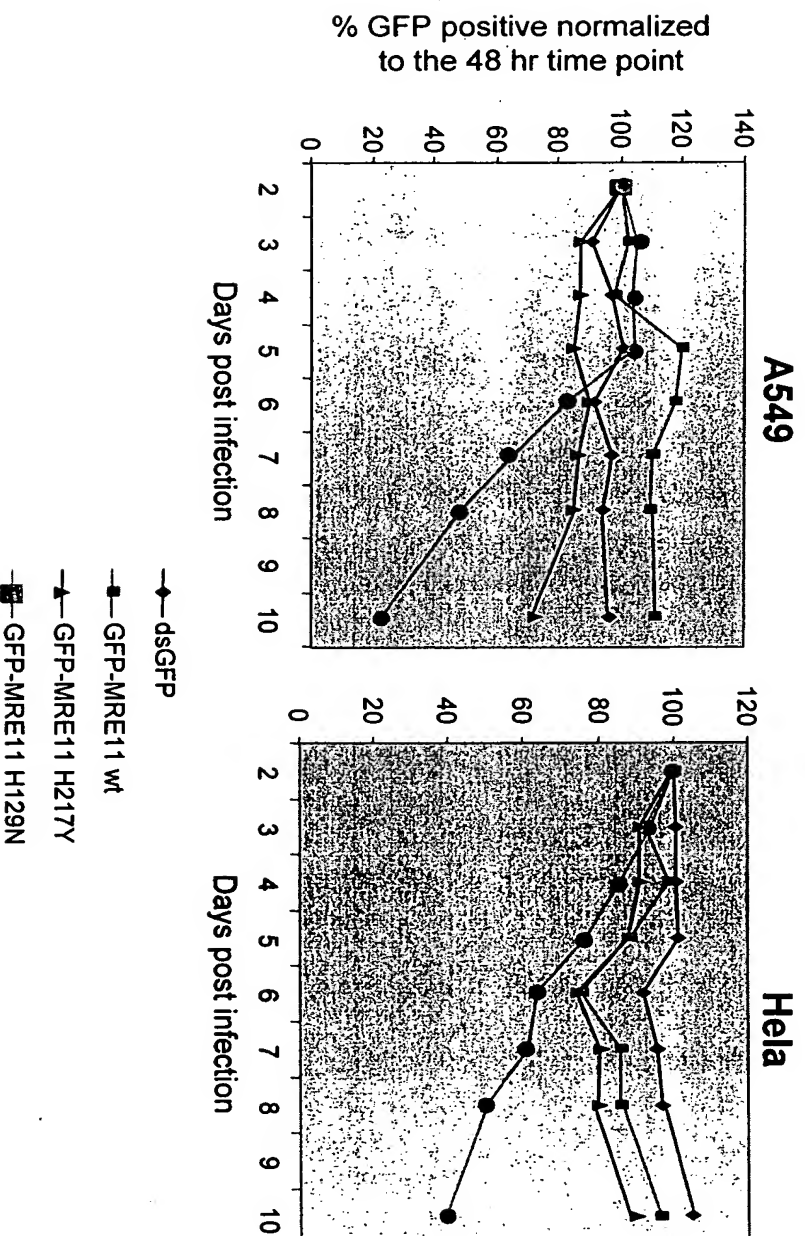


FIG. 8



# Cell Tracker Assay Shows the Activity of GFP-fused MRE11 H129N is Antiproliferative in A549 Cells

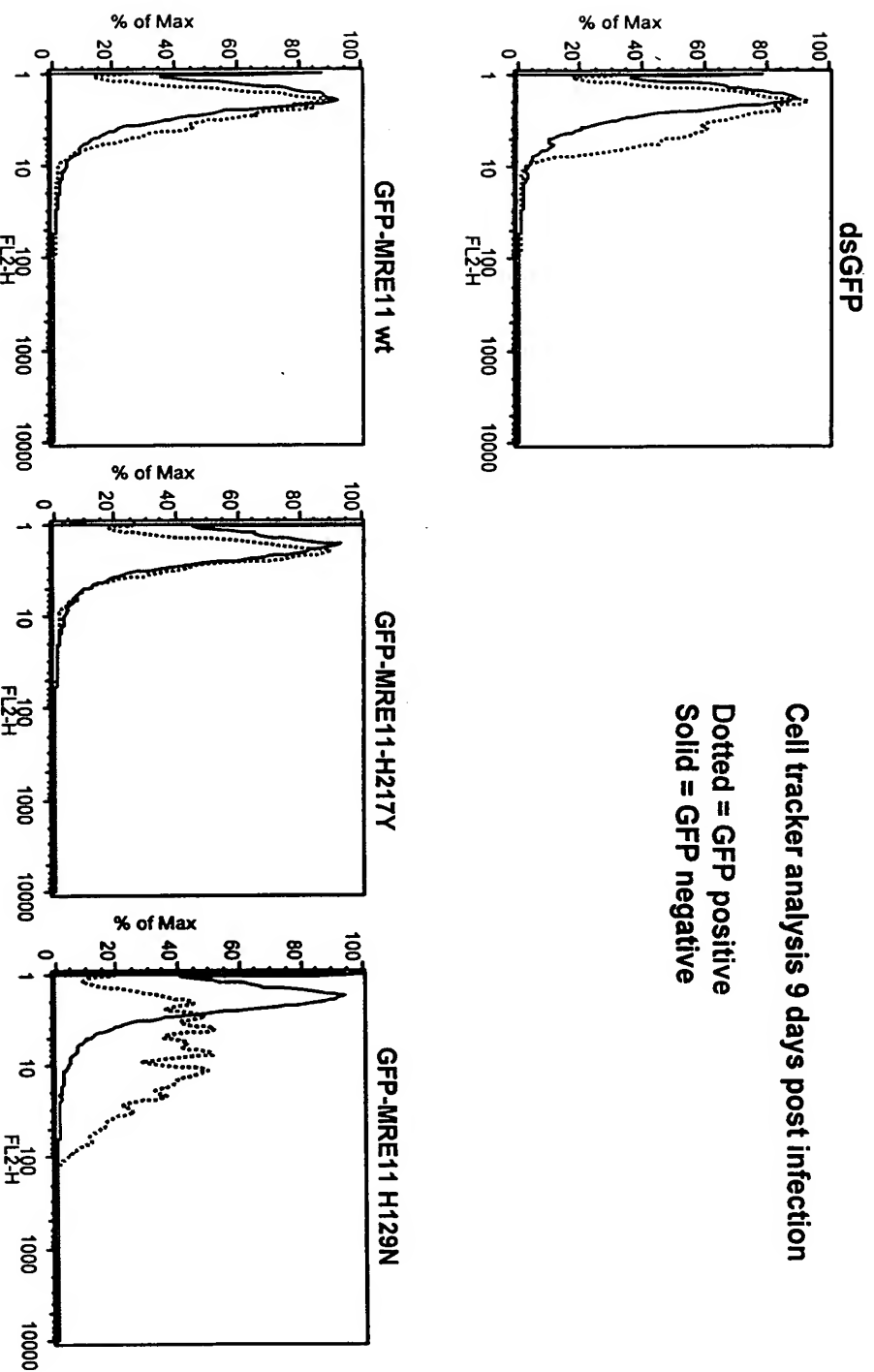
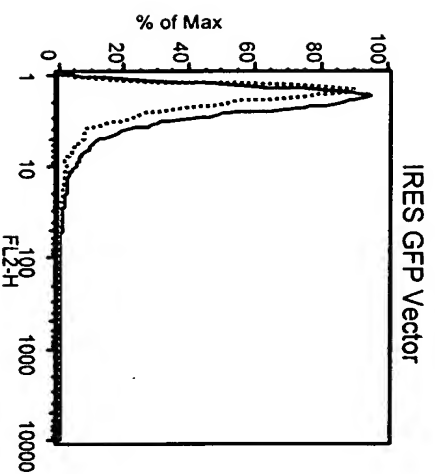


FIG. 9

# Cell Tracker Assay Shows the Activity of MRE11 H129N Using IRES GFP is Antiproliferative in A549 Cells



Cell tracker analysis 9 days post infection

Dotted = GFP positive  
Solid = GFP negative

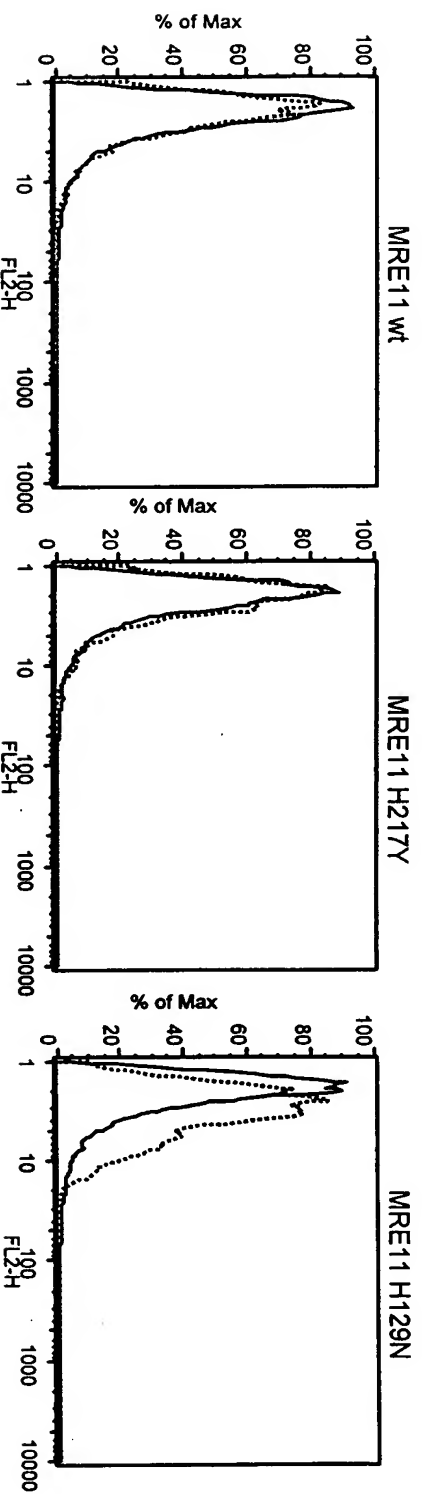


FIG. 10

# Overexpression of GFP-fused MRE11 Wild Type and Mutants in PC-3 and H1299 cells

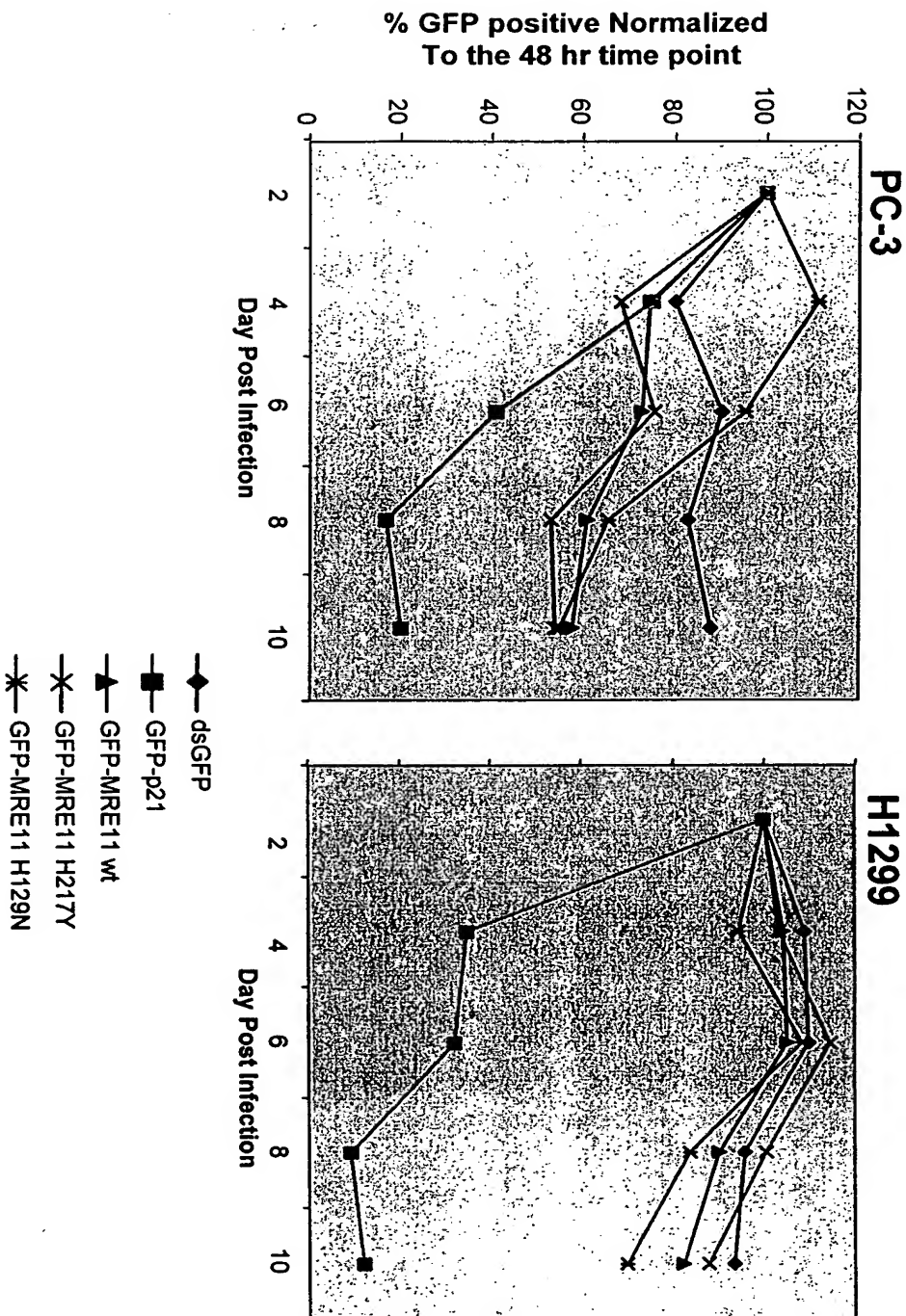


FIG. 11

# Overexpression of MRE11 Wild Type and Mutants is Not Antiproliferative in Normal Cells

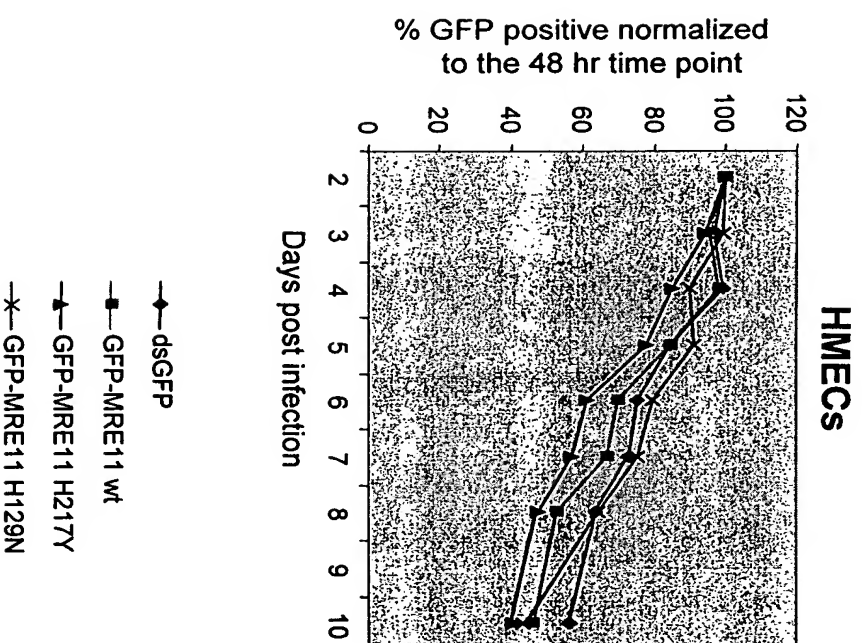
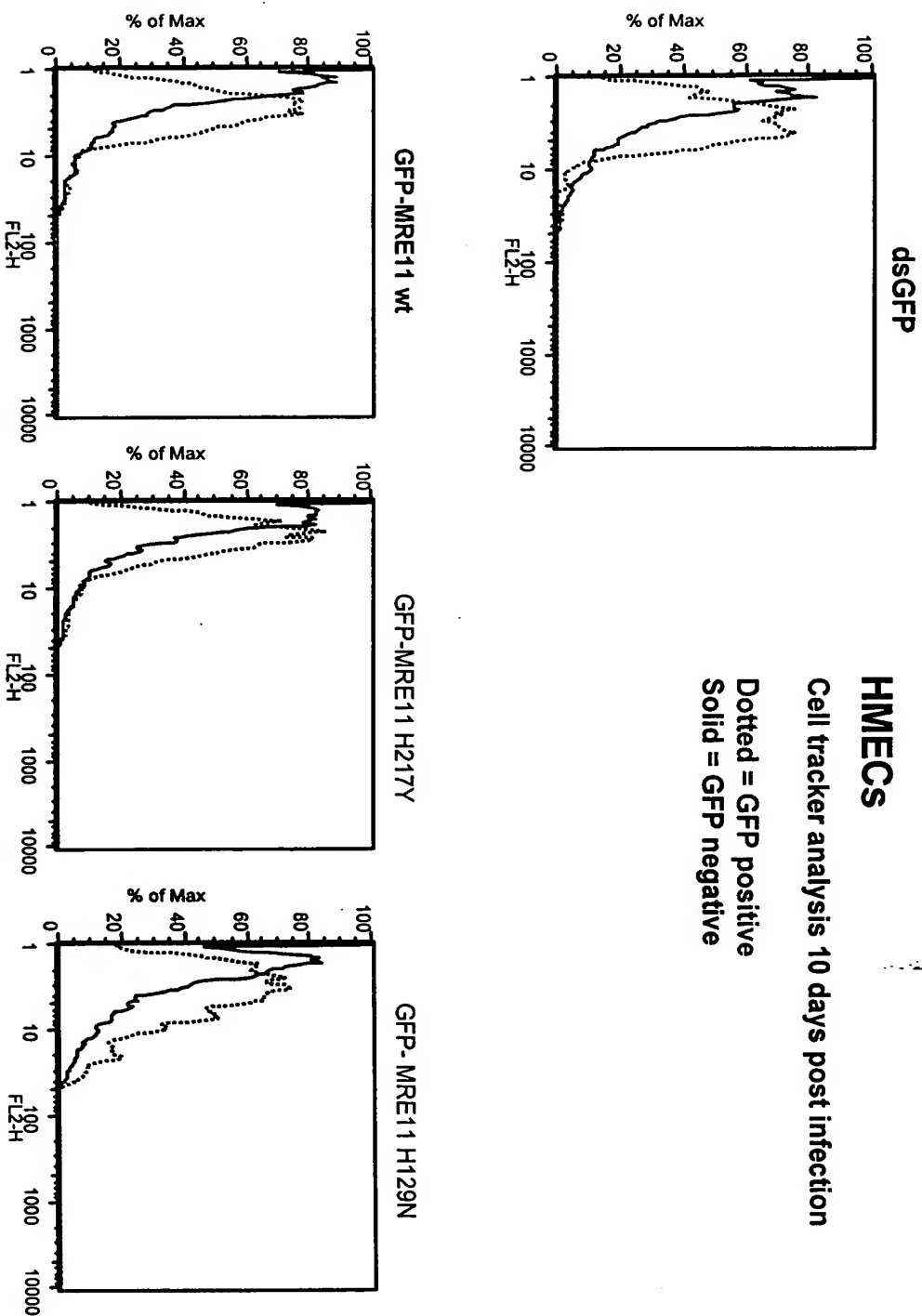


FIG. 12

# No Antiproliferative Activity of MRE11 Wild type or Mutant Proteins is Detected By the Cell Tracker Assay in Normal Cells



**FIG. 13**

# Overexpression of MRE11 Wild Type and Mutants is Not Antiproliferative in Normal Cells

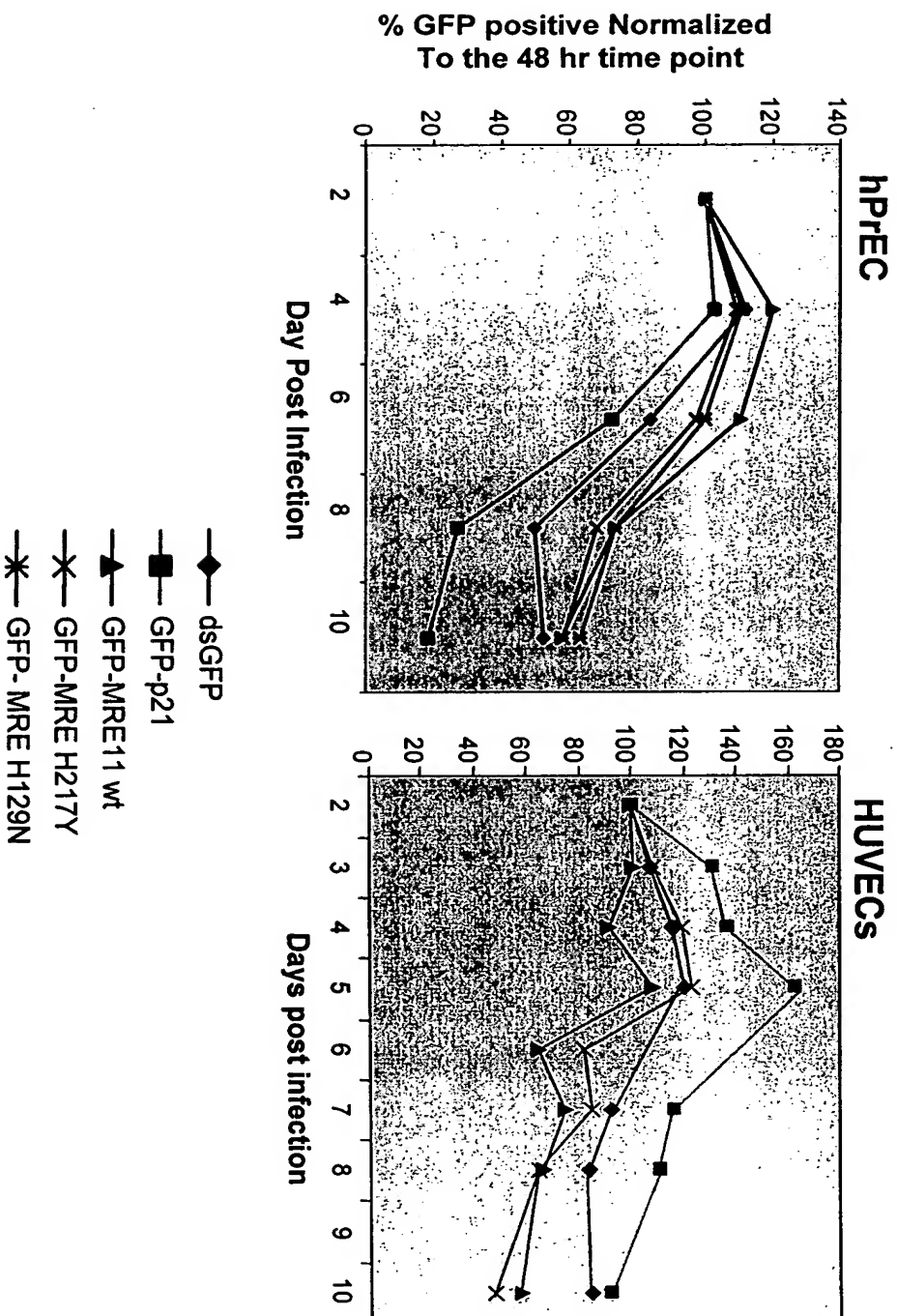


FIG. 14

# MRE11 Specific Antisense Oligo Effects in A549 Cells

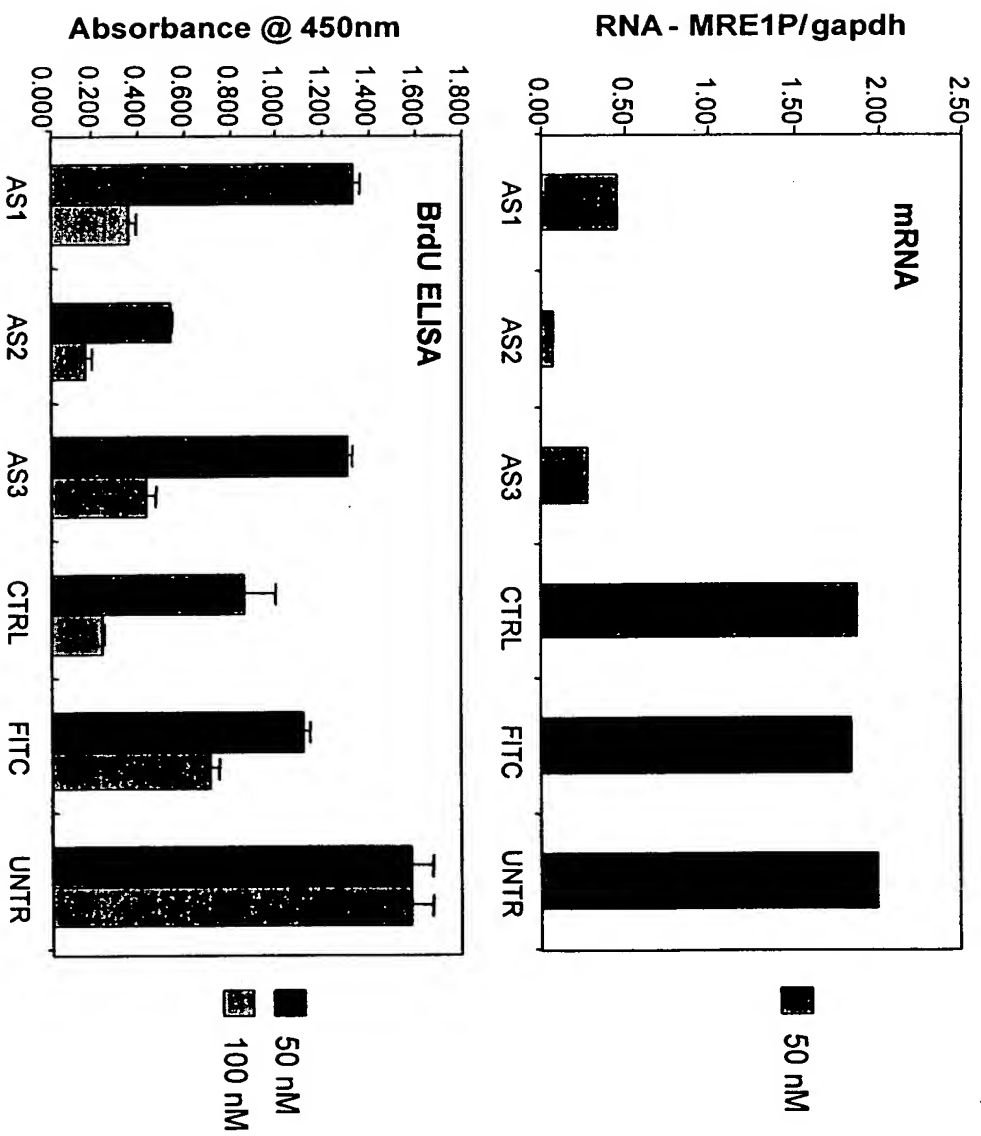


FIG. 15

# Strategies for Assessing Chemosensitization Using Dominant Negative Studies

## Plate based BrdU incorporation ELISA

Hela cells were infected with GFP-fused wt or mutant MRE11

The top 10% GFP positive cells were sorted 5 days after infection

Purified cell populations were plated in 96-well plates for  
chemotherapeutic treatments

BrdU incorporation was measured 48 and 72 after treatment

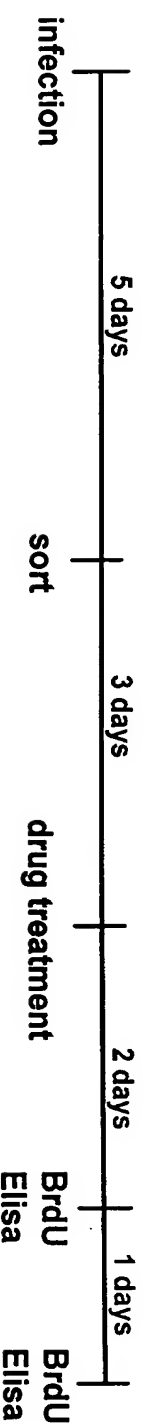


FIG. 16



# Plate-based Chemosensitization Studies of Sorted HeLa Cells Expressing GFP-fused Wild Type or Mutant MRE11

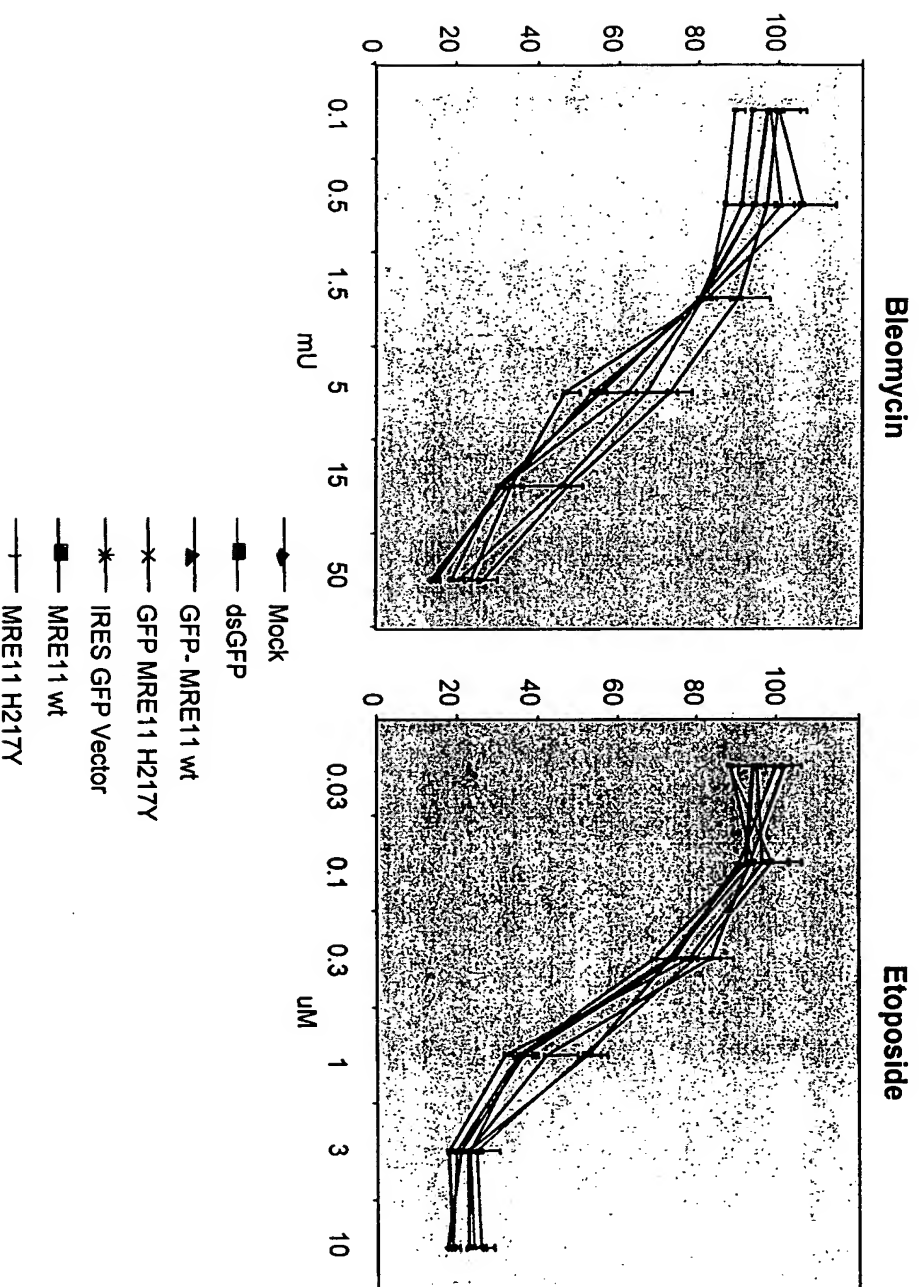


FIG. 17

# Alternative Strategy for Assessing Dominant Negative Chemosensitization Effects

## C II Survival after Drug treatment and wash out (similar to colony survival assay)

GFP positive study to determine selective sensitivity of the GFP positive cells (reflecting cells expressing wild type or mutant proteins) in a mixed population of infected and non-infected cells

- Cells are infected with GFP-fused wt or mutant MRE11
- Cells are then treated with chemotherapeutics for 48 hrs
- After a 48 hr treatment period, the chemotherapeutic is washed out and cells are allowed to recover for 5-8 day.
- After the end of the recovery period, the %GFP positive cells in the treated population relative to the untreated population is assessed by FACS analysis.

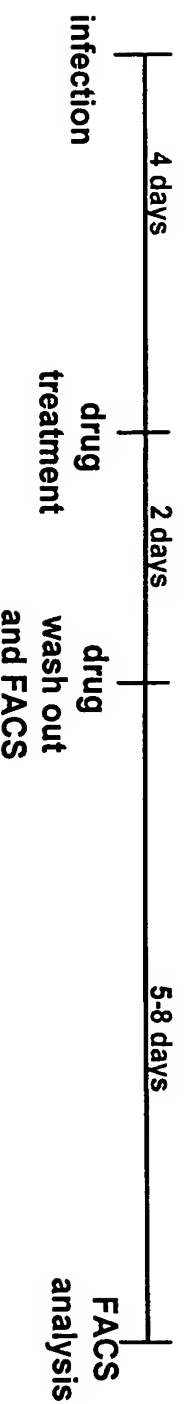
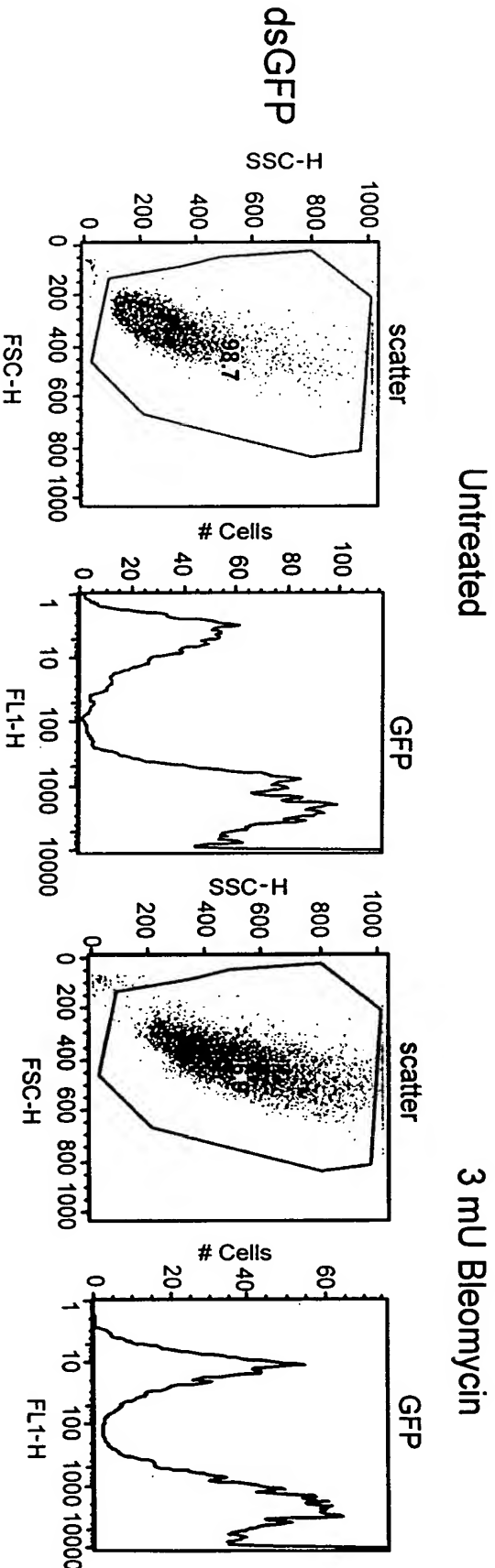


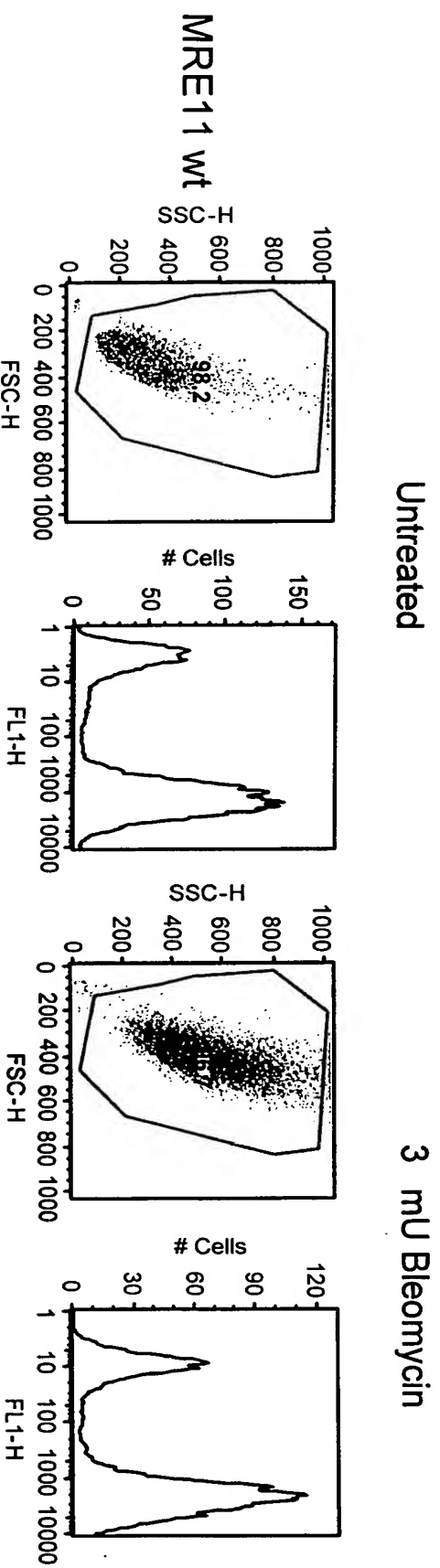
FIG. 18

**No Selective Sensitization of MRE11 Wild Type or Mutant  
Expressing A549 Cells 48 hrs After Bleomycin Treatment**



**FIG. 19A**

**No Selective Sensitization of MRE11 Wild Type or Mutant Expressing A549 Cells 48 hrs After Bleomycin Treatment (cont'd)**



**FIG. 19B**



# A549 Cells Expressing the MRE11 H217Y Mutant Fail to Recover From Bleomycin Treatment

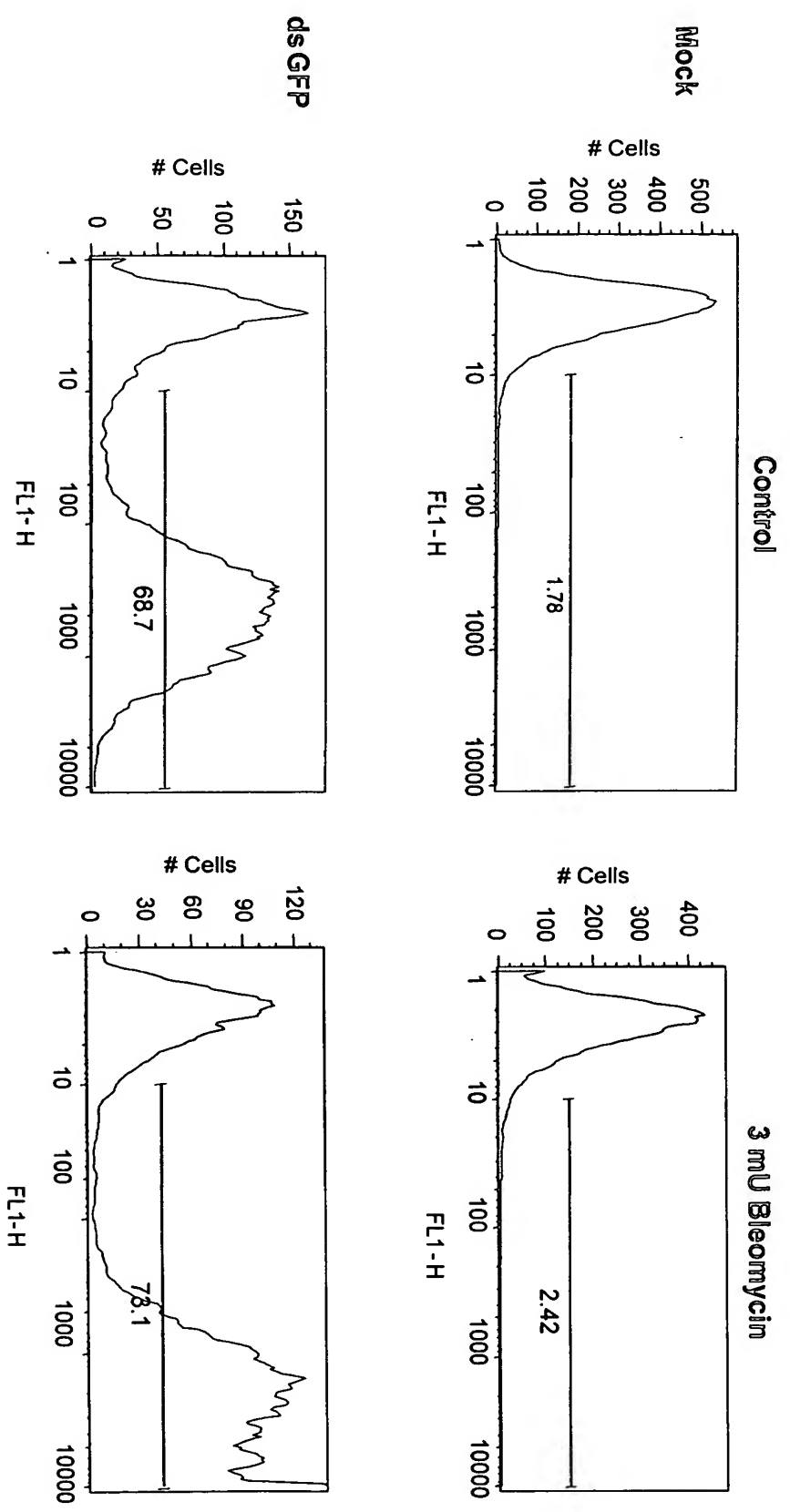


FIG. 20A

# A549 Cells Expressing the MRE11 H217Y Mutant Fail to Recover From Bleomycin Treatment (cont'd)

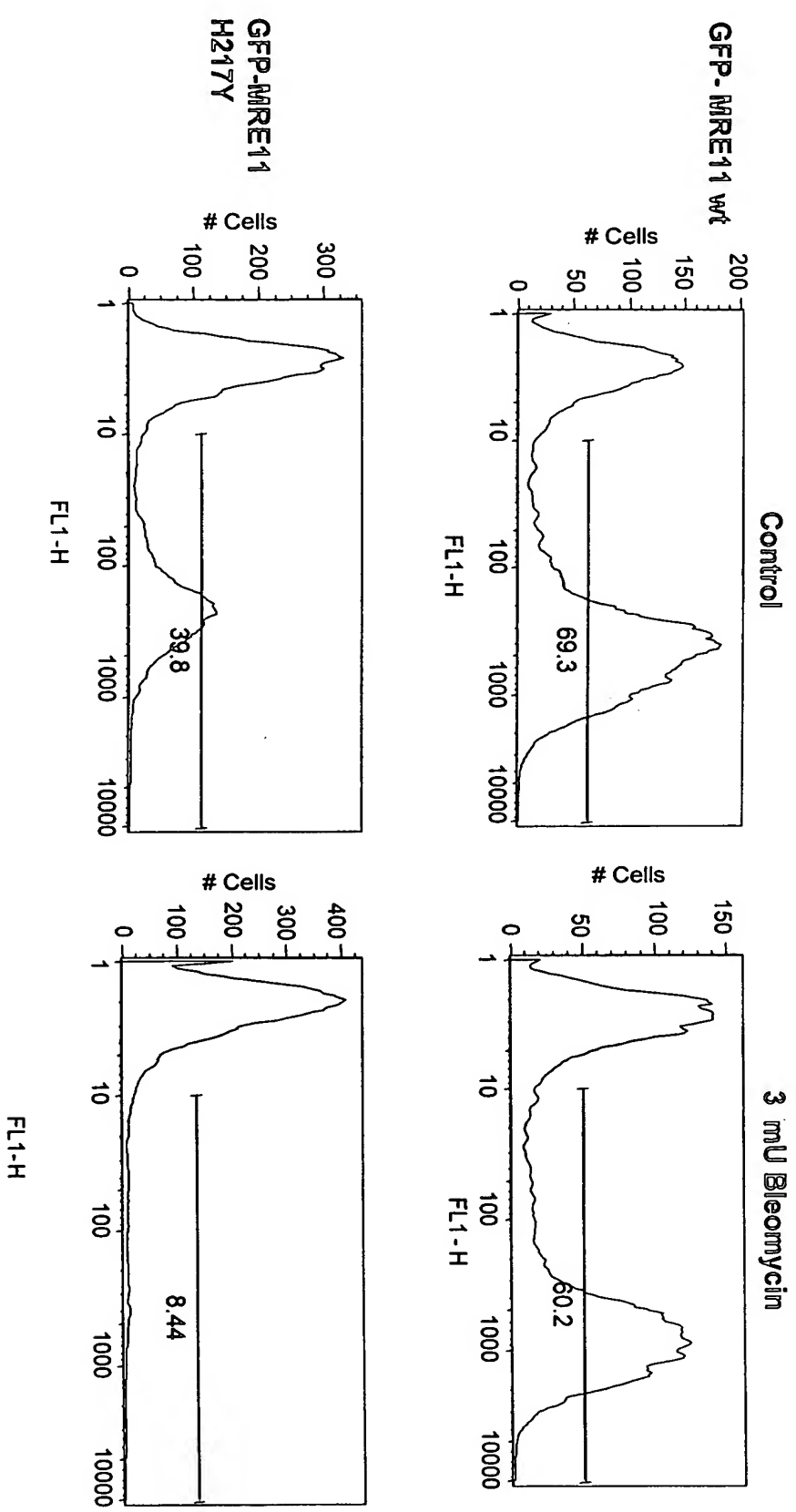


FIG. 20B

# Overexpression of GRP-MRE11 H217Y Mutant in A549 and HeLa Cells Enhances Sensitivity to Bleomycin Treatment

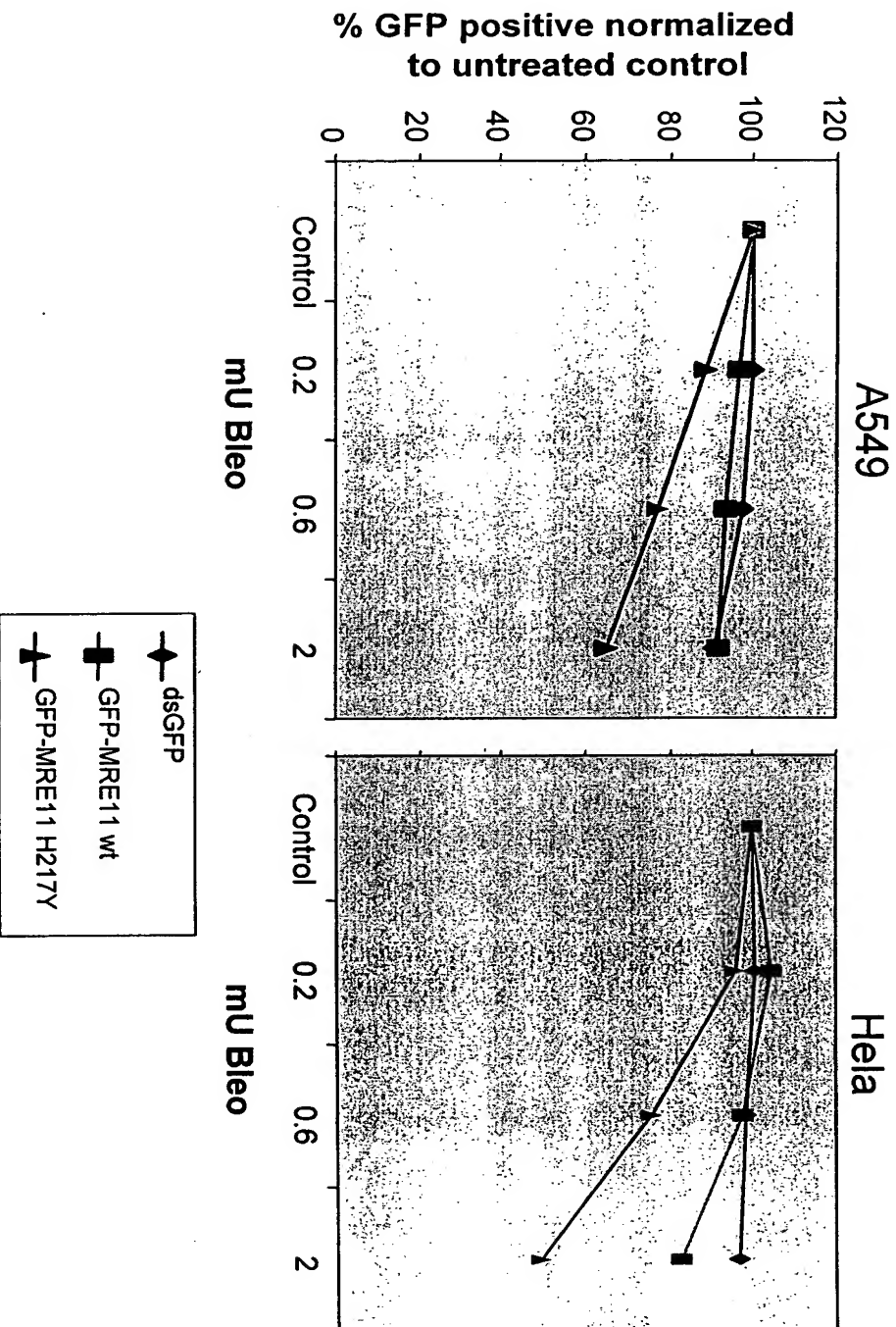


FIG. 21



# Overexpression of GFP-MRE11 H217Y Mutant in Normal HMECs does not Enhance Sensitivity to Bleomycin Treatment

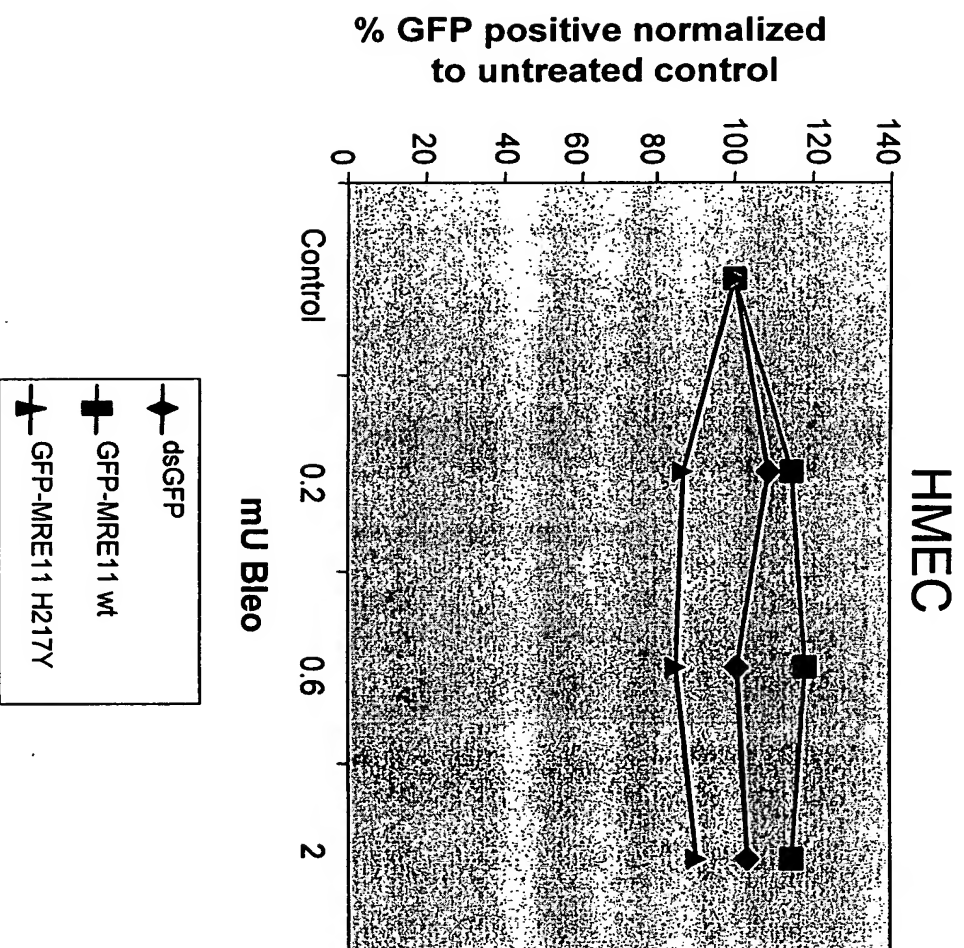
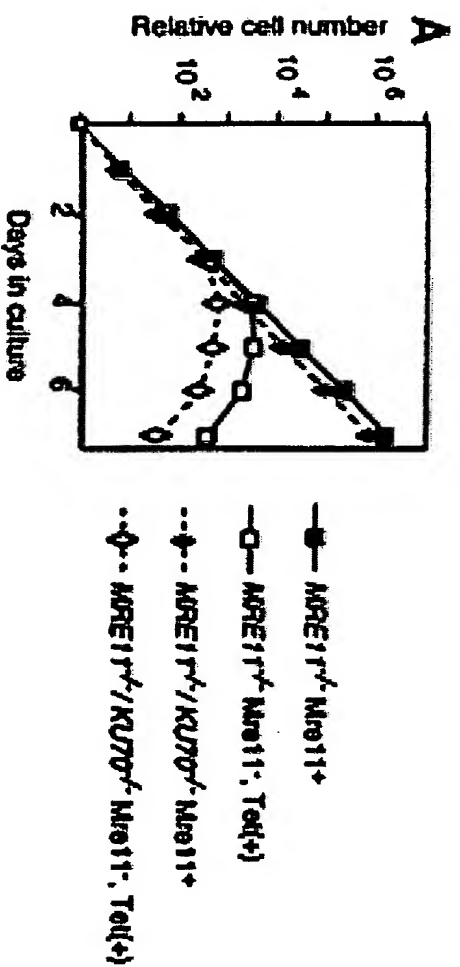


FIG. 22

# Depletion of MRE11 is Antiproliferative in the Hyper-recombinogenic Chicken B-cell line DT40 Made Conditionally Null for MRE11



Additional phenotypes observed in MRE<sup>-/-</sup> cells

- chromosomal aberrations
- centrosome amplification
- enhanced sensitization to ionizing radiation

# **Possible Models Explaining the Antiproliferative and Chemosensitization Effects of MRE11 Inhibition**

**Antiproliferative activity may be explained through  
MRE11's Role in:**

**Double strand break repair**

**Telomeric regulation**

# MRE11 Inhibition May Block Repair of Spontaneous Or Drug Induced Double Strand Breaks

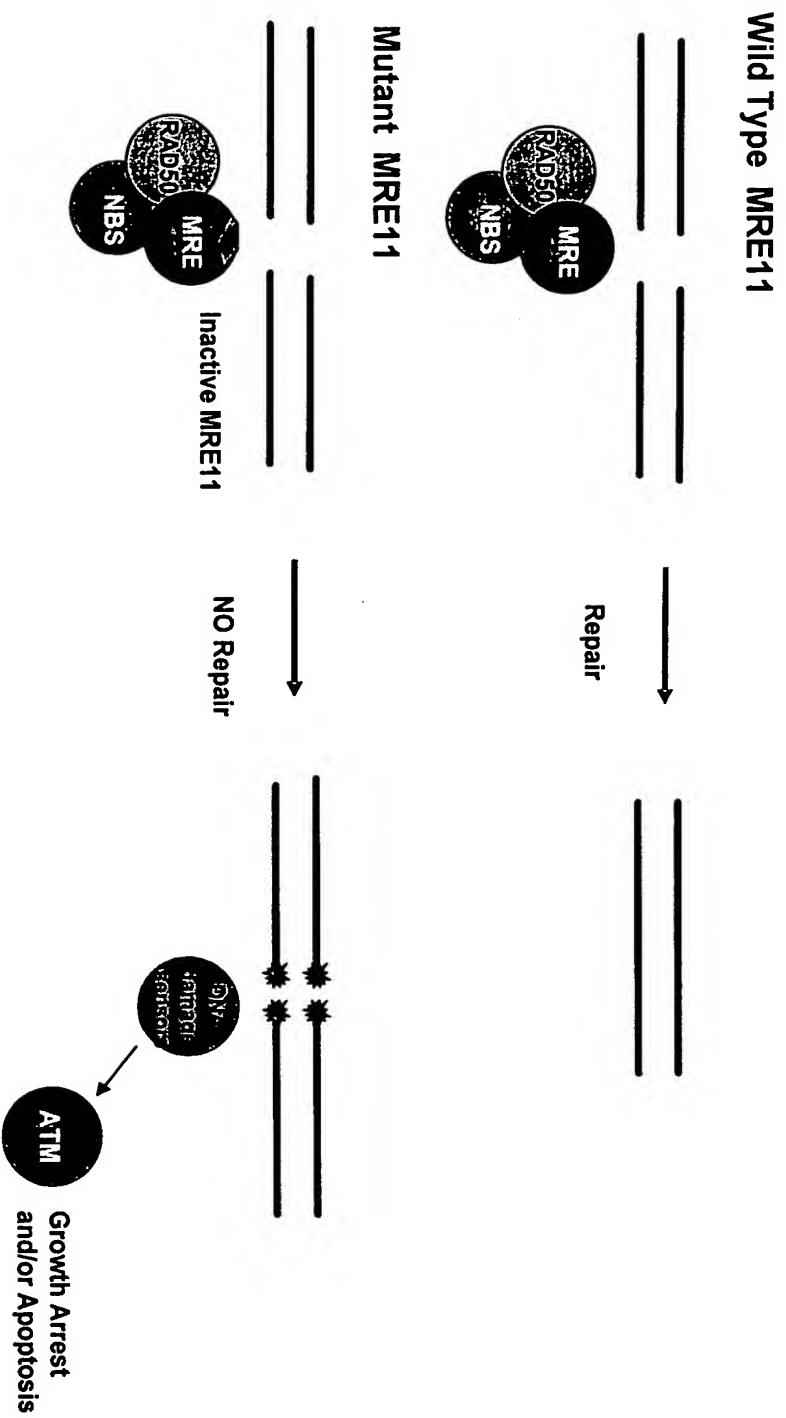


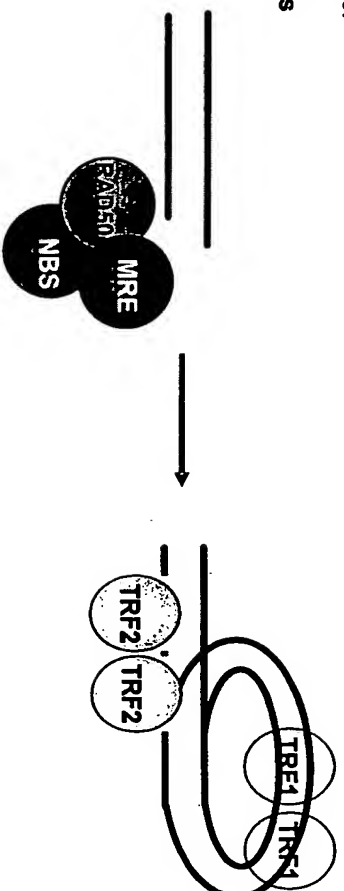
FIG. 25

# MRE11 Inhibition May Block the Formation of the Protective T-loop Structure at Telomere Ends

Wild Type MRE11

Protected telomere end

MRE11 may be required for proper preparation of telomeric DNA for strand invasion that protects telomeric ends



Mutant MRE11

Unprotected telomere end

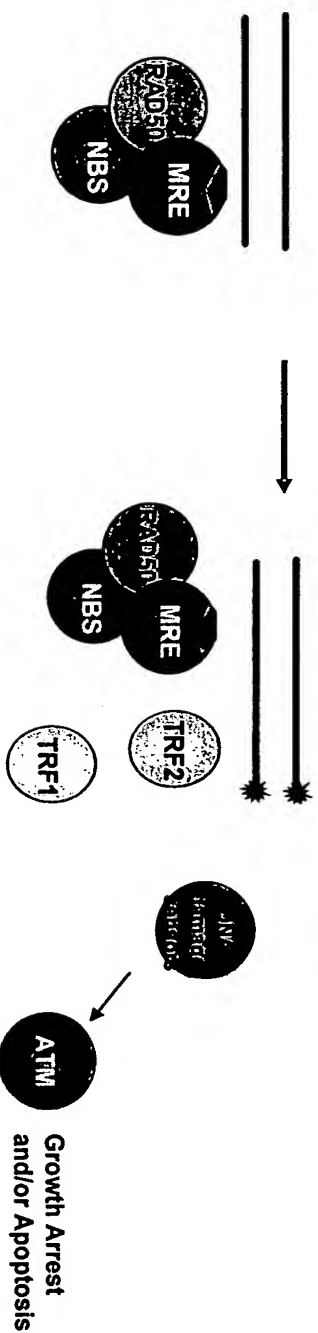


FIG. 26

# MRE11 Summary

## Functional Studies

Source: YTH- PCNA/Nbs1

### Antiproliferative Activity

- Overexpression of MRE11 H129N mutant protein is antiproliferative in tumor cells, but not in normal cells
- No strong antiproliferative effect is seen in cells expressing MRE11 wild type or H217Y mutant

### Chemosensitization

- Overexpression of MRE11 H217Y mutant enhances sensitivity to chemotherapeutic treatment in tumor cells
- Sensitization by the H129N mutant cannot be assessed because of the inherent antiproliferative activity seen with expression of this mutant

## Literature

- Numerous studies have suggested that MRE11 plays an important role in DNA damage repair pathway
- Studies on the yeast protein suggest that inhibition of catalytic activity of MRE11 will result in sensitivity to ionizing radiation

## Conclusion

- Functional studies suggest inhibition of MRE11 will selectively inhibit tumor cell growth and enhance the response of tumor cells to DNA damaging agents

FIG. 27

# Proposed HTS Compatible Biochemical Assay for MRE11

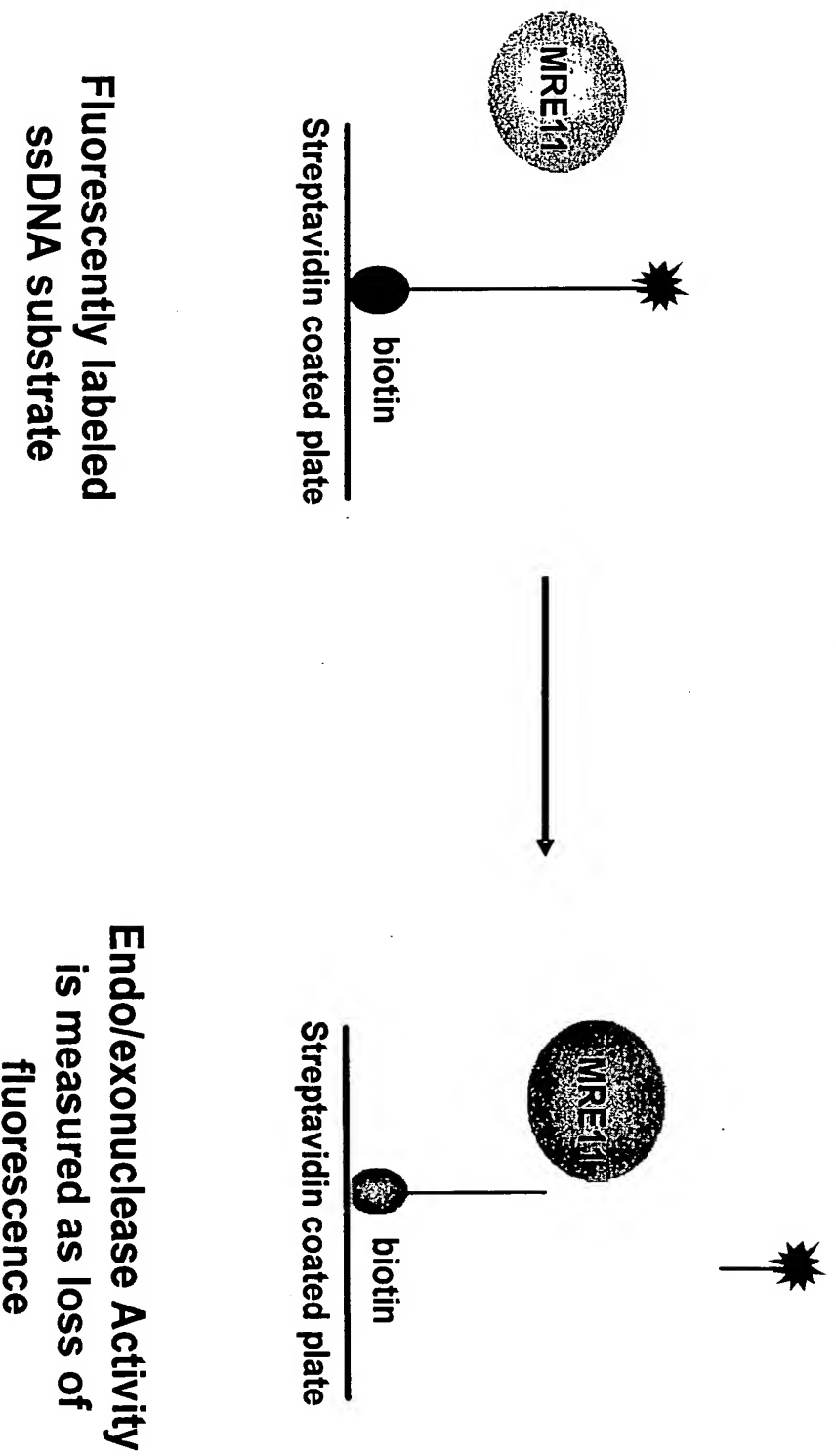


FIG. 28

# Oligonucleotide Duplex Substrate for MRE11 Plate - Based Assay



Sequence was taken from oligonucleotide DG51 (Paul and Gellert, Mol. Cell, 1998), a substrate used to characterize the *in vitro* nuclease activity of recombinant Mre11. A HaeIII cleavage site was incorporated as a positive control for the assay.

FIG. 29



# Biochemical Assay for MRE11 Exonuclease Activity

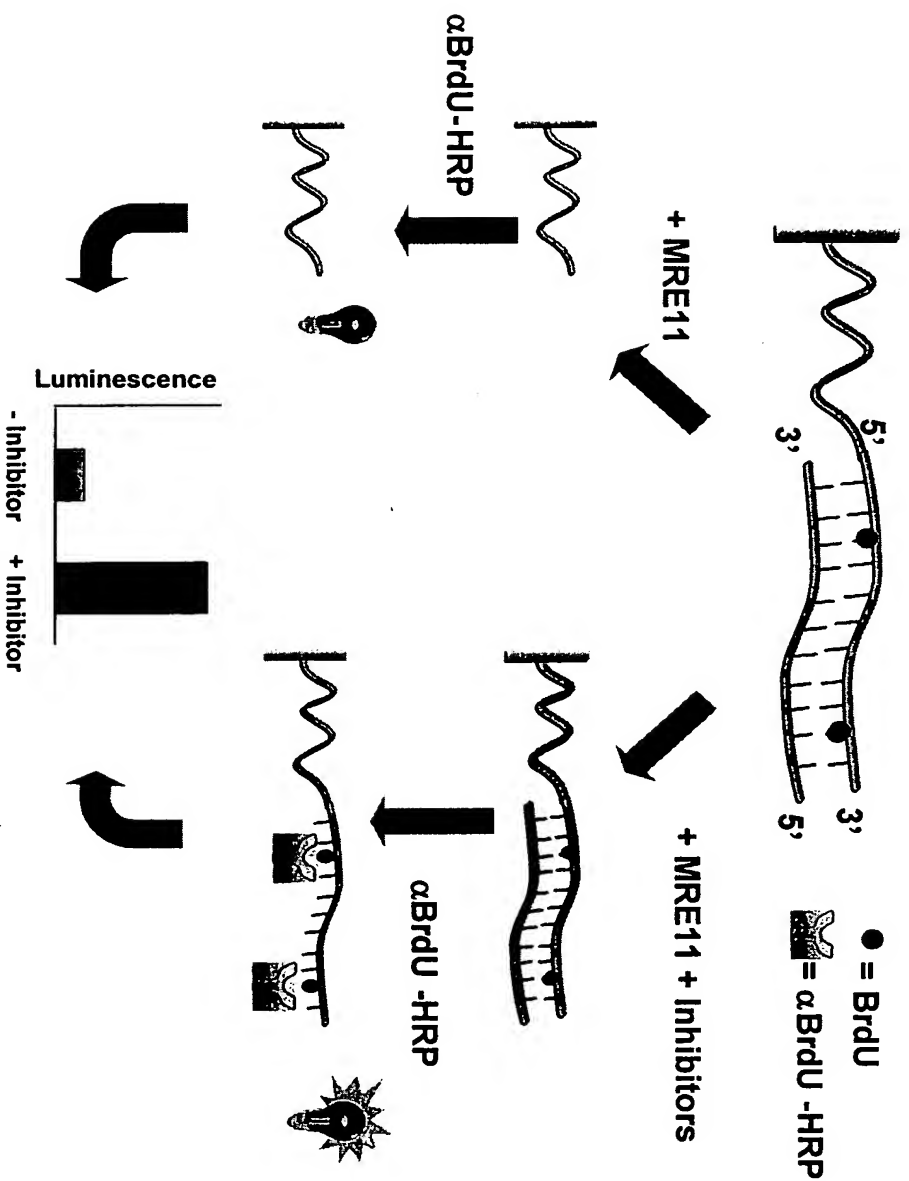


FIG. 30

# Cleavage of Single-stranded Biotinylated Reporter Oligonucleotide by MRE11

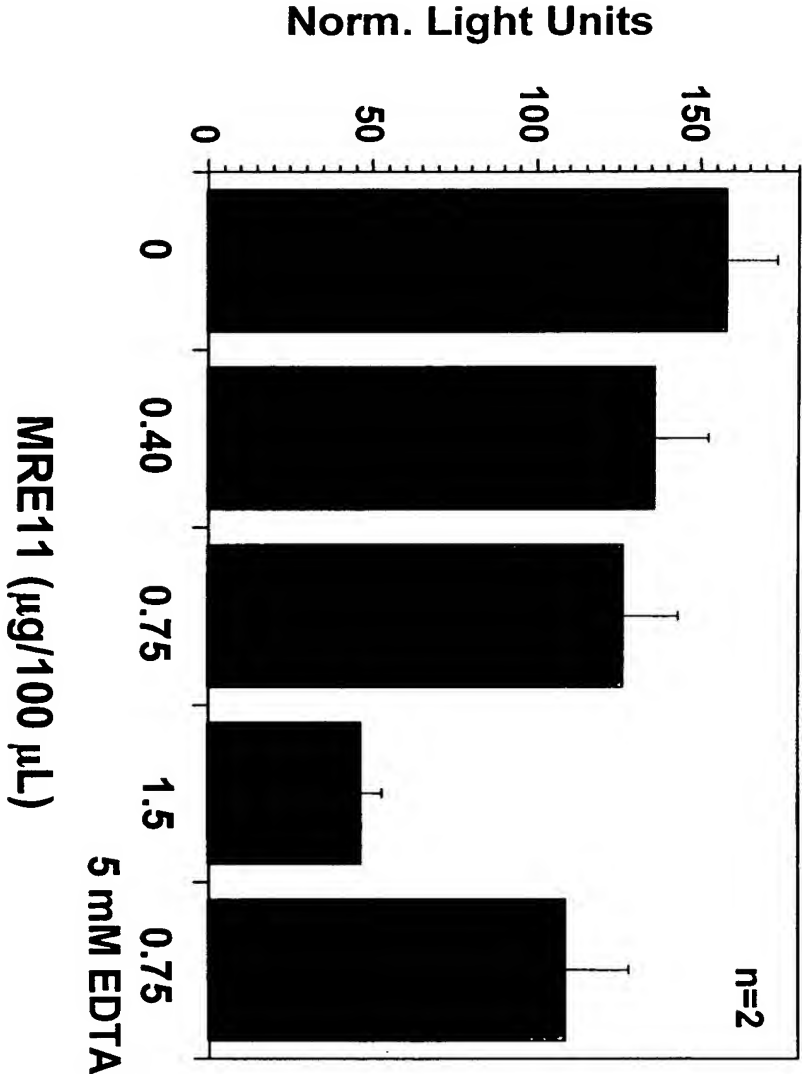
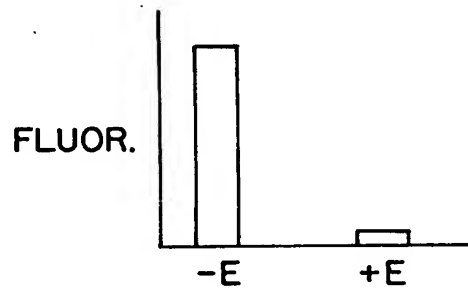
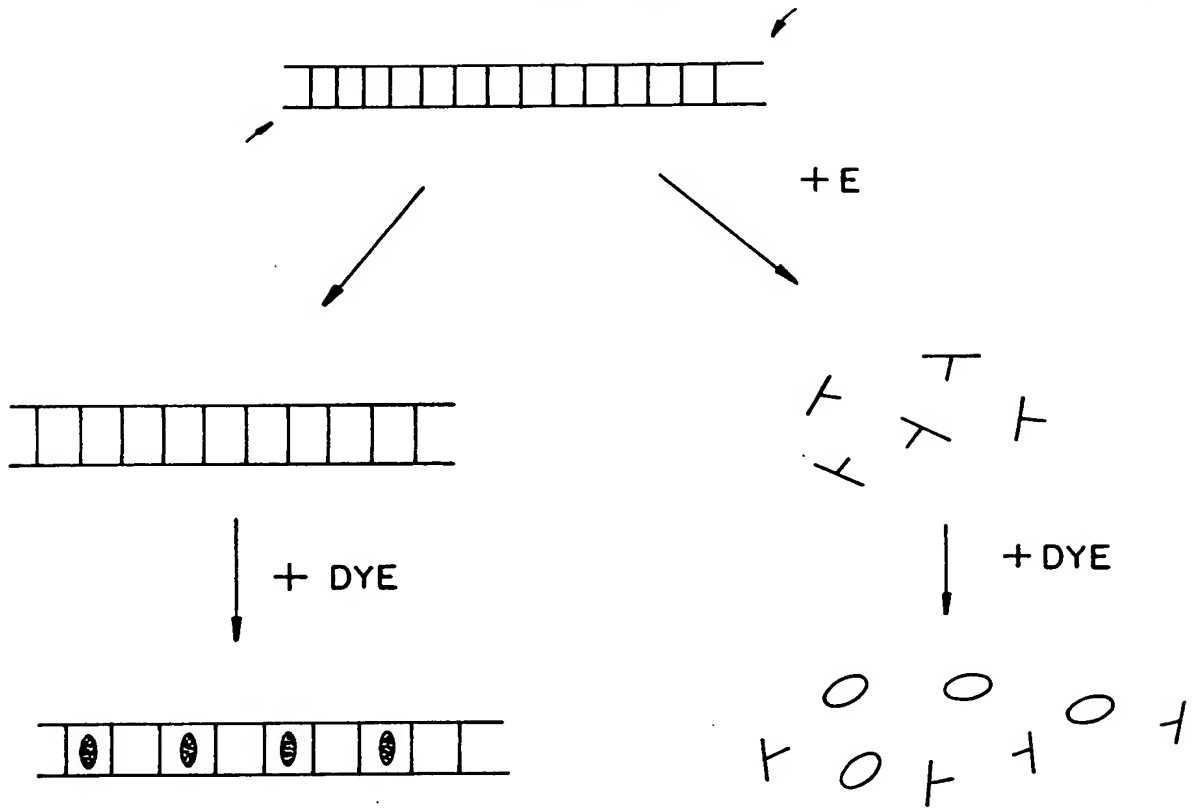


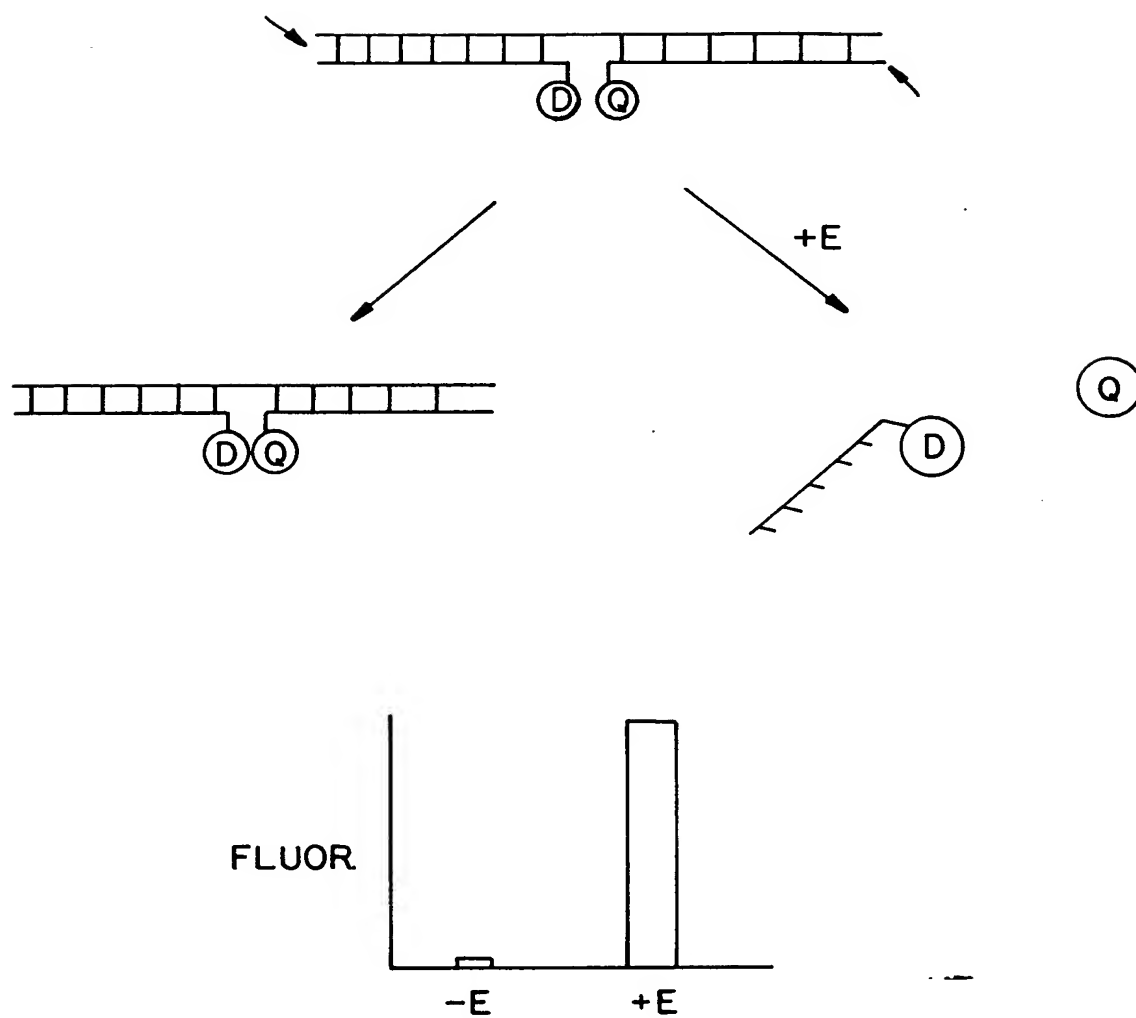
FIG. 31

FIG. 32.



PICOGREEN DYE ASSAY

FIG. 33.



FLUORESCENCE QUENCHING ASSAYS